Identification of LFA-1 as a Candidate Autoantigen in Treatment-Resistant Lyme Arthritis

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Treatment-resistant Lyme arthritis is associated with immune reactivity to outer surface protein A (OspA) of *Borrelia burgdorferi*, the agent of Lyme disease, and the major histocompatibility complex class II allele *DRB1*0401*. The immunodominant epitope of OspA for T helper cells was identified. A homology search revealed a peptide from human leukocyte function–associated antigen-1 (hLFA-1) as a candidate autoantigen. Individuals with treatment-resistant Lyme arthritis, but not other forms of arthritis, generated responses to OspA, hLFA-1, and their highly related peptide epitopes. Identification of the initiating bacterial antigen and a cross-reactive autoantigen may provide a model for development of autoimmune disease.

Lyme disease is a multisystem illness caused by infection with the spirochete *Borrelia burgdorferi* (*1*). A prominent late manifestation of the disease is Lyme arthritis (*1, 2*). About 10% of patients with Lyme arthritis develop what we have termed antibiotic treatment–resistant Lyme arthritis, which typically affects one knee for months to years after multiple courses of antibiotics (*1*). Such patients have no detectable spirochetal DNA in joint fluid after antibiotic therapy, which suggests that the spirochete has been eliminated by this treatment (*3*). Because there is increased frequency of the *HLA-DRB1*0401* allele in these patients (*4*), an autoimmune etiology should be considered. The hypervariable 3 region (HVR3) at residues 67 to 74 of *DRB1*0401* is associated with susceptibility to rheumatoid arthritis (RA) and is contained in at least 15 different *DRB1* alleles (*5*). Most patients with prolonged treatment-resistant Lyme arthritis have one of these homologous alleles (*4*). What antigen are these class II molecules presenting?

Borrelia burgdorferi induces an immune response of expanding reactivity to an array of spirochetal proteins over months to years (*6*). Antibody reactivity to outer surface protein A (OspA) typically develops near the beginning of prolonged episodes of arthritis (*7*). T cell lines from patients with treatment-resistant Lyme arthritis preferentially recognize OspA, compared with patients with treatment-responsive disease. OspA-reactive type 1 T helper (T_H1) cells are detectable in the synovial fluid of individuals with treatment-resistant arthritis years after antibiotic treatment (*7*). Thus, these patients may have progressed into an autoimmune state by developing a cross-reactive response between OspA and a self-antigen.

We used the DRB1*0401 peptide-binding algorithm (*8*) to determine the scores for all nine-residue peptides in the OspA protein sequence that contained an appropriate pocket 1 anchor residue—F, I, M, L, T, V, or Y—necessary for binding in the DRB1*0401 peptide-binding cleft. According to this algorithm, only peptides with scores greater than 2 are likely to bind and be able to be presented by the DRB1*0401 molecule (*8*). The highest scoring peptide that was identified, $OspA_{165–173}$, had a predicted binding score of 6.5, and the next best scoring peptide, $OspA_{237–245}$, achieved a score of 3.7. To verify that these peptides can bind to DRB1*0401 in vitro, the binding of ^{125}I-labeled m1–7 (YRAMATL; predicted DRB1*0401 binding score = 5.9), which has the consensus binding motif for DRB1*0401 (*9*), was measured when in competition with unlabeled 20-residue peptides from OspA. Only $OspA_{154–173}$, which contains the DRB1*0401-predicted dominant epitope $OspA_{165–173}$, inhibited binding of the radiolabeled peptide m1–7 to purified DRB1*0401 (Table 1), confirming the algorithm's prediction.

To test for T cell reactivity in vivo, we made use of class II–deficient mice transgenic for a chimeric DRB1*0401 molecule (DRB1*0401-tg) (*9*). Any $CD4^+$ T cell response generated in these mice can be directly attributed to the presence of the DRB1*0401 molecule. The ElisaSpot assay was used for measuring anti-

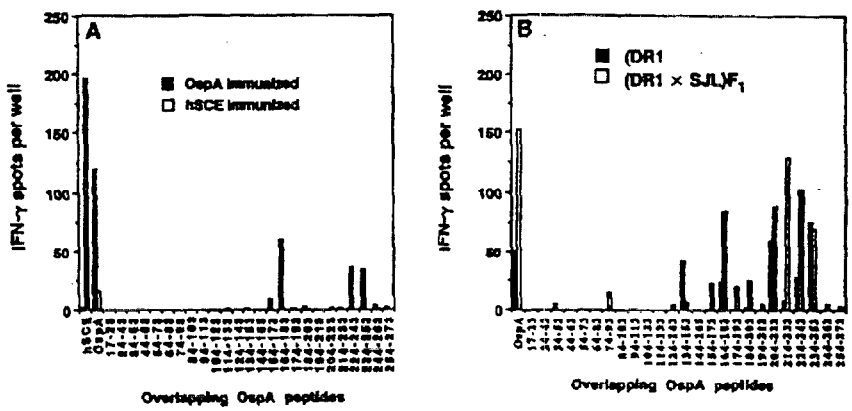

Fig. 1. IFN-γ ElisaSpot analysis demonstrates $OspA_{165–173}$ as the functional, immunodominant epitope of OspA in DR4-tg mice. (**A**) Class II–deficient, DRB1*0401-tg mice, immunized with whole OspA, but not a control protein, recall whole OspA and $OspA_{164–183}$ specifically. DRB1*0401-tg mice were immunized in both hind footpads with either 50 μl of OspA (44 μg/ml) or human spinal chord extract (hSCE; 100 μg/ml) in complete Freund's adjuvant. Eight days later, draining popliteal lymph nodes were isolated and 5×10^5 cells were cultured with either a positive control stimulant, CD3 antibody, mAb 145.2C11, or one of the following test antigens: hSCE (50 μg/ml), OspA (10 μg/ml), overlapping OspA 20-mer peptides (10 μg/ml each), or medium alone. IFN-γ production was analyzed 24 hours later by ElisaSpot (*10*). Values from wells with medium alone were subtracted from values from wells that contained antigen. Antigens are listed as overlapping 20-mer peptides spanning OspA, beginning with amino acid 17. Residues 1 to 17 contain the leader sequence and are therefore cleaved during export through the bacterial membrane. Representative experiments of six OspA-immunized and two hSCE-immunized mice are shown. (**B**) DRB1*0101-tg and (DRB1*0101-tg × SJL)F_1 mice immunized with whole OspA recall $OspA_{165–173}$ as well as other epitopes. In contrast to the DRB1*0401-tg mice, the DRB1*0101-tg mice express murine class II; therefore, a broader array of OspA epitopes is recognized. Experiments were performed as described above. One of three and one of two representative experiments are shown for DRB1*0101-tg and (DRB1*0101-tg × SJL)F_1 mice, respectively.

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gen-specific T cell reactivity, a sensitive and efficient technique that allows detection of cytokine production at the single cell level, which may occur in the absence of proliferation (*10*). We initially assayed for production of T_H1 and T_H2 cytokines, interferon-γ (IFN-γ), and interleukin-5 (IL-5), respectively. Both IFN-γ–producing and IL-5–producing cells were detected when cells were activated with a polyclonal stimulus [anti-CD3; monoclonal antibody (mAb) 145.2C11]. In contrast, when cells were stimulated with OspA antigen, IFN-γ production was dominant, with essentially no detectable IL-5 secretion (*11*). Therefore, detection of IFN-γ was used as the readout for antigen-specific T cell reactivity in all subsequent assays. DRB1*0401-tg mice were immunized with OspA and lymph node cells were stimulated with overlapping 20-residue peptides of OspA: the immunostimulatory epitopes correlated precisely with the epitopes predicted by the DRB1*0401 algorithm (Fig. 1A). Immunization of the DRB1*0401-tg mice with $OspA_{165–173}$ resulted in a recall response to whole OspA in vitro (*11*). Hence, we have identified the immunodominant epitope of OspA in the context of DRB1*0401. To test the ability of $OspA_{165–173}$ to be presented by *DRB1* alleles related to DRB1*0401 (*5*), we performed the same experiment in mice transgenic for DRB1*0101 (*12*). These transgenic mice possess a full complement of murine class II genes, thereby providing distinct major histocompatibility complex (MHC) alleles for OspA peptide presentation. ElisaSpot analyses of OspA-immunized DRB1*0101-tg or (DRB1*0101-tg × SJL)F_1 mice showed reactivity to $OspA_{165–173}$ as well as to an array of other epitopes (Fig. 1B). In contrast to DRB1*0401-tg mice, reactivity toward $OspA_{165–173}$ developed as a subdominant epitope, suggesting that alternative determinants are available for binding that could influence disease development. Interestingly, the F_1 mice had a response to $OspA_{165–173}$ that was three times the response of DRB1*0101-tg mice. This is likely because of expression of the murine I-Eβ^s chain, which is homologous in the HVR3 to DRB1*0401 (*5*), thereby providing twice the number of class II molecules for presentation of this particular peptide. Thus, we have identified the immunodominant OspA peptide recognized in the context of DRB1*0401 and found that DRB1 and murine class II alleles homologous to DRB1*0401 in their HVR3 can also present this epitope.

We searched the Genetics Computer Group gene bank for human proteins containing sequences homologous to $OspA_{165–173}$. Of the 20 peptides retrieved with the highest identity and homology scores, two were of human origin: hLFA-1 (CD11a/CD18, integrin $\alpha_L\beta_2$) and 40*S* ribosomal protein. Only the peptide contained in hLFA-1, hLFA-$1\alpha_{L332–340}$, attained a significant DR4-binding score (7.3), with six–amino acid identity (YVIEGT*SKQ*; nonconserved residues in italics), suggesting hLFA-1 as a potential autoantigen. The peptide contained within the 40*S* ribosomal protein sequence (YVLEG*KELF*) attained a DR4-binding score of 0, mostly because of Lys at position p6, which is not tolerated in the DR4-HVR3 (*13*). The hLFA-$1\alpha_{L332–340}$ peptide is located extracellularly in the interactive or I-domain that mediates the binding interaction between LFA-1 and its ligand, intercellular adhesion molecule–1 (ICAM-1) (*14*). When the DR4-binding algorithm was applied to the entire I-domain (amino acids 170 to 349), hLFA-$1\alpha_{L332–340}$ achieved the highest predicted bind-

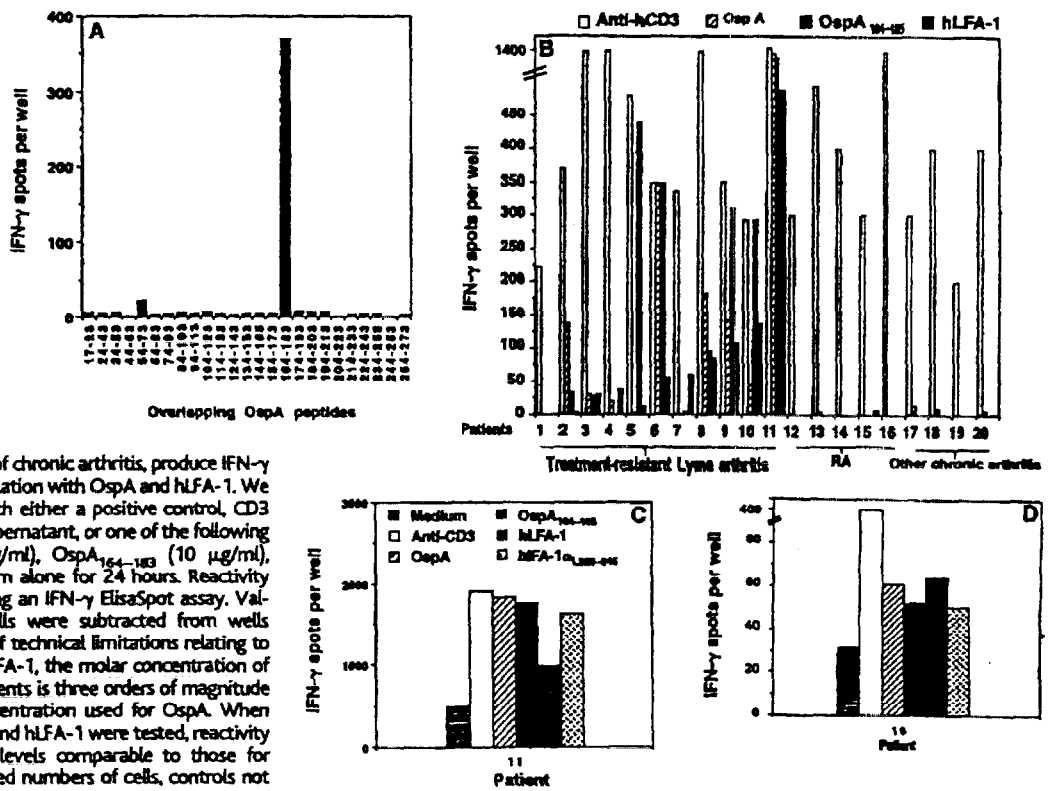

Fig. 2. SF T cells from patients with treatment-resistant Lyme arthritis generate a response to hLFA-1. (**A**) IFN-γ ElisaSpot analysis of 3 × 10^5 SF T cells per well, from patient 4, cultured with each of the overlapping OspA peptides at 10 μg/ml, revealed $OspA_{164–183}$ as the immunodominant epitope (*10*, *15*). Reactivity to whole OspA was positive as determined by proliferation assay (medium, 2552 177 248 cpm; OspA, 24,497 177 2079 cpm) (*16*). (**B**) SF T cells from patients with treatment-resistant Lyme arthritis, but not other forms of chronic arthritis, produce IFN-γ in response to in vitro restimulation with OspA and hLFA-1. We cultured 3 × 10^5 SF cells with either a positive control, CD3 antibody hybridoma OKT3 supernatant, or one of the following test antigens: OspA (10 μg/ml), $OspA_{164–183}$ (10 μg/ml), hLFA-1 (70 ng/ml), or medium alone for 24 hours. Reactivity was determined by performing an IFN-γ ElisaSpot assay. Values from medium-alone wells were subtracted from wells containing antigen. Because of technical limitations relating to the purification process of hLFA-1, the molar concentration of hLFA-1 used in these experiments is three orders of magnitude lower than the optimal concentration used for OspA. When equimolar amounts of OspA and hLFA-1 were tested, reactivity to OspA was depressed to levels comparable to those for hLFA-1 (*11*). Because of limited numbers of cells, controls not tested for reactivity to $OspA_{164–183}$ were patients 17, 18, and 20; and, for hLFA-1, patients 17 and 18. (**C**) Treatment-resistant Lyme arthritis patient 11, who is homozygous for *DRB1*0401*, demonstrates SF T cell reactivity to the 20-mer containing the OspA homologous, *DRB1*0401*-defined dominant epitope within the I-domain, hLFA-$1\alpha_{L326–345}$. We cultured 3 × 10^5 SF cells with hLFA-$1\alpha_{L326–345}$ (25 μg/ml). IFN-γ ElisaSpot assay was performed as described above. (**D**) Treatment-resistant Lyme arthritis patient 10, who is heterozygous for an RA-associated allele (*DRB1*0102*), demonstrates SF T cell reactivity to the 20-mer containing the OspA homologous, *DRB1*0401*-defined dominant epitope within the I-domain, hLFA-$1\alpha_{L326–345}$. We cultured 3 × 10^5 SF cells with equimolar amounts of $OspA_{164–183}$, hLFA-1, and hLFA-$1\alpha_{L326–345}$. IFN-γ ElisaSpot assay was performed as described above.

ing score (7.3), nearly twice that of the next highest scoring peptide, hLFA-1$\alpha_{L196-204}$ (binding score = 4.3), and higher than that of OspA$_{165-173}$. We determined, by performing the peptide binding competition assay [median inhibitory concentration (IC_{50}) = 0.7825 mM], that hLFA-1$\alpha_{L331-345}$, a 15-mer containing the core residues 332 to 340, was capable of binding DRB1*0401 in vitro.

To test the hypothesis that hLFA-1 is an autoantigen in patients with treatment-resistant Lyme arthritis, but not in other forms of chronic inflammatory arthritis, we mapped the immunodominant epitope of OspA in synovial fluid (SF) cells from a patient (4) with treatment-resistant Lyme arthritis (Fig. 2A) (*10*). As in the DRB1*0401-tg mouse, OspA$_{164-183}$ was immunodominant. We then analyzed the antigen reactivity profile of SF T cells from patients with treatment-resistant Lyme arthritis as well as patients with other forms of chronic arthritis (*15*). ElisaSpot for IFN-γ production (*10*) and proliferation assays (*16*) showed that people in a panel consisting of only those with treatment-resistant Lyme arthritis have varying degrees of SF T cell reactivity to whole OspA, OspA$_{164-183}$ as well as hLFA-1 (Fig. 2B). Reactivity to hLFA-1 is due to recognition of hLFA-1$\alpha_{L326-343}$, the region homologous with OspA$_{164-183}$ (Fig. 2, C and D). This reactivity appears to develop over time, as patients who initially showed no response to hLFA-1 had marked reactivity when tested 1 to 3 months later (*11*).

Borrelia burgdorferi sensu stricto is the only spirochetal strain associated with treatment-resistant Lyme arthritis (*17*) and the sole strain that contains the OspA$_{165-173}$ sequence that is highly related to hLFA-1$\alpha_{L332-340}$. Murine LFA-1a differs significantly from hLFA-1 at this particular epitope, providing an explanation for why chronic Lyme arthritis does not develop in DRB1*0401-tg mice exposed to *B. burgdorferi* (*12*).

Our demonstration of autoreactivity against hLFA-1 (in particular, the predicted cross-reactive epitope) in patients with treatment-resistant Lyme arthritis suggests that this disease involves an autoimmune process. However, although the genetic predisposition for development of treatment-resistant Lyme arthritis has been correlated with DR4, we cannot rule out other genetic, environmental, and infectious factors that might be involved. As mentioned above, the HVR3 of the DRB1 chains associated with RA possesses a shared epitope at residues 67 to 74 (*5*). Most patients with severe RA carry at least one allele that contains the shared epitope sequence of DRB1*0401, henceforth referred to as an RA-associated allele (*5*). Individuals who develop the most severe form of RA typically have two RA-associated alleles (*18*). HLA typing of our panel of 11 treatment-resistant Lyme arthritis patients revealed that 7 possessed at least one RA-associated allele (*15*), and 9 made a response to hLFA-1. Patient 11, who was homozygous for DRB1*0401, responded four times more vigorously to both OspA and hLFA-1 than the next highest responder. In patients with other forms of arthritis, the presence of an RA-associated allele by itself was not sufficient for induction of an OspA or hLFA-1 response, as at least five of the nine control patients possessed an RA-associated allele (*15*) yet made no response to OspA or hLFA-1. Thus, priming by *B. burgdorferi* infection or at least with OspA may be required for development of an autoimmune response to hLFA-1. Other factors may also be involved in development of treatment-resistant Lyme arthritis, as some treatment-resistant patients who do not possess an RA-associated allele make a response to hLFA-1 and some patients with treatment-resistant Lyme arthritis do not respond to either OspA or hLFA-1 (Fig. 2B).

On the basis of our DRB1*0401-restricted OspA T cell epitope mapping data, as well as previous work on immune reactivity and cytokine production in response to infection with *B. burgdorferi* (*7*), we propose a model on how an immune reaction to *B. burgdorferi* might result in development of an autoimmune response against hLFA-1: *B. burgdorferi* enters the host via a tick bite and disseminates to multiple tissues. Months later, a highly inflammatory immune response develops in the joint, and this response is dominated by T_H1 IFN-γ–producing cells that contain OspA reactive cells. We propose that the high local concentration of IFN-γ up-regulates expression of ICAM-1 (*19*) on synoviocytes and synovial fibroblasts as well as of MHC class II molecules on the local professional and nonprofessional antigen-presenting cells (APCs) (*19*). This enhanced ICAM-1 expression leads to recruitment of LFA-1 expressing cells, in particular activated T_H1 cells. The combination of elevated LFA-1 expression on T cells and macrophages plus MHC class II up-regulation on APCs may result in increased LFA-1 peptide presentation by macrophages and synoviocytes that have processed either endogenous or phagocytosed LFA-1 (*20*). Hence, a vicious cycle is initiated so that, even after elimination of the spirochetes by antibiotic therapy, the OspA-primed T cells remain activated by stimulation with LFA-1. The release of inflammatory cytokines by these activated T cells and macrophages may then result in tissue damage and joint destruction (*21*).

Table 1. Inhibition of m1–7 peptide binding to DRB1*0401 (*15*) by 20-residue peptides of OspA.

OspA peptide*	IC_{50} (μM)	Nine-residue peptides with appropriate p1 anchor residue†	DR4-algorithm scores for peptides with an appropriate p1 anchor residue‡
154–173	4.381	161, 162, 165, 166	(−) 0.4, (−) 0.8, (+) 6.5, (−) 5.4
54–73	>100	54, 55, 58, 61, 63	(−) 1.1, (−) 4.1, (−) 6.3, (−) 2.8, (−) 0.1
74–93	>100	75, 76, 79, 86	(−) 6.6, (+) 1.1, (+) 2.4, (−) 1.9
124–143	>100	126, 132, 136, 137	(−) 4.7, (−) 4.3, (−) 3.3, (−) 2.4

*Testing was limited to peptides with both sufficient quantity of material and a broad range of DR4-predicted binding scores. OspA$_{164-183}$ was not available for testing. †The number of potential DRB1*0401-binding 9-residue peptides contained within a 20-residue sequence was determined by the presence of an appropriate p1 anchor residue (F, I, L, M, V, T, or Y). p1 anchor residue amino acid numbers are listed for each candidate peptide. ‡Scores were calculated for OspA nine-residue peptides beginning with F, I, L, M, V, T, or Y (9). Scores are listed, respectively, for each 9-mer peptide contained within the 20-mer peptide tested.

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8. J. Hammer *et al.*, *J. Exp. Med.* 180, 2353 (1994); K. W. Marshall *et al.*, *J. Immunol.* 154, 5927 (1995). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
9. In vitro binding studies were performed as described, and generation of DR4-tg mice are documented in [K. Ito *et al.*, *J. Exp. Med.* 183, 2635 (1996)]. Overlapping 20-mer OspA peptides were synthesized by R. Woods and were a generous gift from M. Hanson (Medimmune, Gaithersburg, MD). OspA 15-mer (SYVLEGTLTAEKTTL) and 9-mer (YVLEGTLTA) peptides, as well as hLFA$_{\alpha}$ 15-mer (IYVIEGTSKQDLTSF) and mLFA-1 15-mer (IYAIEGTNRQDLTSF) peptides were purchased from Bio-Synthesis. The hLFA$_{\alpha}$ 20-mer (ELQKKIYVIEGTSKQDLTSF) was purchased from Research Genetics.
10. Single-cell suspensions of popliteal lymph node cells from immunized mice, or Ficoll-Hypaque (Sigma) centrifugation isolation of human lymphocytes from peripheral blood mononuclear cells or SF, were prepared and coincubated with appropriate antigen [5 × 10^5 cells per well (mouse) or 3 × 10^5 cells per well (human) and OspA or OspA peptides (10 μg/ml), hLFA-1 (70 ng/ml), or anti-CD3 supernatant] to T-

Spot plates (Autoimmune Diagnostika) precoated with capture monoclonal antibody to IFN-γ (4 μg/ml) and blocked with Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Plates were washed at 24 hours and probed with a sandwich biotinylated antibody to IFN-γ. Spots were detected with an anti-biotin alkaline phosphatase (AP) (murine ElisaSpot) or streptavidin–horseradish peroxidase (human ElisaSpot) with detection enzyme reactions of either NBT/BCIP (Pierce) or 3-amino-9-ethylcarbzole and *N,N*-dimethylformamide (Pierce/Fisher), generating purple or red spots, respectively. Scores were determined by the Series I T-Spot Image analyzer (Autoimmune Diagnostika) as the difference between the number of spots produced with and without antigen. OspA protein was a kind gift from B. Lade and J. Dunn (Brookhaven National Lab) and purified hLFA-1 was a kind gift from D. Staunton (ICOS Corporation). Human spinal chord extract was prepared according to standard procedures. The following antibodies were used for murine in vitro assays: 145.2C11 (murine antibody) or OKT3 (human antibody), CD3 antibody (hybridoma supernatant); R4-6A2, coat, IFN-γ antibody and XMG1.2, capture, biotinylated IFN-γ antibody (PharMingen); biotin-AP antibody (Vector). The following antibodies were used for human in vitro assays: OKT3, CD3 antibody (hybridoma supernatant); coat, IFN-γ antibody, and capture, biotinylated IFN-γ antibody (Endogen); streptavidin–horseradish peroxidase (Zymed).

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12. B10.m/Sn mice transgenic for DRB1*0101 were a kind gift from D. Zaller (Merck Research Laboratories); S. Feng, S. W. Barthold, L. K. Bockenstedt, D. M. Zaller, E. Fikrig, *J. Infect. Dis.* 172, 286 (1995).
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15. We studied 11 patients (7 male, 4 female; between 12 and 40 years old) with treatment-resistant Lyme arthritis and 9 control patients (4 male, 5 female; between 17 and 78 years old) with RA or other forms of chronic inflammatory arthritis. All Lyme patients met the case definition of the U.S. Centers for Disease Control and Prevention for diagnosis of Lyme disease. They had arthritis affecting the knee and serologic reactivity with *B. burgdorferi* by ELISA and protein blotting. The 11 Lyme arthritis patients and 5 of the control patients were evaluated in the Lyme Disease Clinic at New England Medical Center (NEMC). The remaining 3 RA (patients 12, 13, and 15) and 1 psoriatic (patient 19) control patients' samples were a generous gift from R. Schumacher (Department of Medicine, University of Pennsylvania Medical School). The protocol was approved by the Human Investigations Committee, and informed consent was obtained from each subject. Patients with Lyme arthritis were treated with both oral and intravenous antibiotic regimens. The duration of arthritis after antibiotic therapy ranged from 2 to 33 months. High-resolution HLA-DR typing with sequence-specific amplification was performed by the Clinical Laboratory of Immunology (NEMC) and by Lee Ann Baxter-Lowe (University of South Carolina, Columbia, SC). Patient *DRB1* alleles are as follows: 10, *0102* and *1501*; 6, *0102* and *1501*; 5, *0401* and *1501*; 7, *0701* and *1601*; 2, *0301* and *1201*; 1, *1* and *11*; 4, *14* and *15*; 11, *0401* and *0401*; 8, *0402* and *7*; 9, *0301* and *1302*; 3, *0404* and *13*; 12, *0401* and *1*; 13, *15* and *7*; 16, *4*; 15, *0401* and *7*; 17, *4* and *17*; 18, *11*, *3*, or *13*; 20, *1* and *13*. Insufficient DNA was available from patients 14 and 19, so *DR* typing was not performed on them.
16. Patient SF cells were plated in 96-well U-bottomed plates (Costar) at a density of 2×10^5 cells per 200 μl in complete RPMI medium (Sigma). Cells were stimulated for 5 days with antigen (2 days with phytohemagglutinin), pulsed with 0.5 μCi of [^{3}H]thymidine during the final 16 to 18 hours, and harvested for scintillation counting. Insufficient cells were available from patients 5 and 11; therefore proliferation assays were not performed. All Lyme arthritis patients' cells responded to OspA (except for patient 1) and $OspA_{164-182}$ (except for patients 1 and 2). Responses ranged from 254 to 2552 cpm (background) and from 2275 to 56,725 cpm (antigen).
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Pioneer Axon Guidance by UNC-129, a *C. elegans* TGF-β

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The *unc-129* gene, like the *unc-6* netrin gene, is required to guide pioneer motoraxons along the dorsoventral axis of *Caenorhabditis elegans*. *unc-129* encodes a member of the transforming growth factor–β (TGF-β) superfamily of secreted signaling molecules and is expressed in dorsal, but not ventral, rows of body wall muscles. Ectopic expression of UNC-129 from ventral body wall muscle disrupts growth cone and cell migrations that normally occur along the dorsoventral axis. Thus, UNC-129 mediates expression of dorsoventral polarity information required for axon guidance and guided cell migrations in *C. elegans*.

Axon guidance along the dorsoventral (D/V) axis of animals of diverse phyla involves secreted, laminin-related, UNC-6/netrin guidance cues (*1*). The signaling pathways activated by these molecules require the UNC-5 and UNC-40/DCC transmembrane receptor families (*2–4*). In *C. elegans*, mutations in *unc-129* (*5*) cause defects in the dorsally oriented trajectories of motoraxons that resemble those present in *unc-5*, *unc-6*, and *unc-40* mutants (*5*, *6*).

A 6.5-kb genomic subclone of cosmid C53D6 was able to rescue the uncoordinated phenotype of *unc-129* mutants after germline transformation (*7*, *8*) (Fig. 1A). Sequence analysis by the *C. elegans* genome-sequencing consortium (*9*) revealed a single open reading frame on this fragment that encodes a protein related to the TGF-β superfamily. The corresponding 1.5-kb cDNA (*10*) includes 5 exons, 34 base pairs (bp) of 5′ untranslated region (UTR), and 281 bp of 3′ UTR and is predicted to encode a protein of 407 amino acids (Fig. 1B). Northern (RNA) analysis of wild-type mRNA revealed a single transcript (*11*) consistent with the size of the cDNA. The 6.5-kb rescuing genomic fragment includes 3 kb of 5′ promoter sequence. A minigene containing 4.5 kb of 5′ promoter sequence fused to the *unc-129* cDNA was able to rescue the phenotype of *unc-129* mutants, indicating that there are no essential regulatory elements in introns or the 3′ sequence (*12*).

UNC-129 shares features with the TGF-β superfamily, including a signal sequence, a prodomain, and a COOH-terminal region that contains seven conserved cysteines (*13*). The UNC-129 COOH-terminal sequence identity ranges from 33% with human BMP-7 to 24% with TGF-β2. Thus, *unc-129* likely represents a subfamily of the TGF-β superfamily.

Sequence analysis revealed the absence of residues in UNC-129 that would be expected between the α-helical region and β sheet of TGF-β molecules (Fig. 1C) (*14*). This interdomain region forms a β turn with a protruding loop accessible to solvent. The three-dimensional structures of TGF-β1 and TGF-β2 differ at this site, which may promote their differing receptor-binding affinities (*15*). Deletion of the loop in TGF-β1 abolishes certain TGF-β1–mediated responses (*16*). Without knowledge of the crystal structure of UNC-129, it remains unclear whether the missing residues form the COOH-terminal end of the long α-helix or affect receptor specificity.

In *C. elegans*, TGF-β signaling pathways

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SAFETY/EFFICACY CONCERNS RE: LYME VACCINE: LYMErix

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BACKGROUND:

Lyme disease is a multi-systemic infection caused by infection with the spirochetal bacteria, Borrelia Burgdorderi. It is the most common vector borne illness in the United States. It is one of the leading infectious diseases in the United States. The actual incidence of lyme disease is unknown, but it is known that the reported statistics which are based on surveillance criteria, underreports the actual incidence of the disease by excluding known cases which do not meet the criteria. Surveillance criteria are used simply to get a sense of the rate of growth of an infection, not the true incidence of it.

A recent study in NEJOM reported that doctors routinely fail to report even cases that meet the surveillance criteria because of cumbersome reporting procedures and a distaste for paperwork. That study estimated this underreporting would result in 10 times more cases being reported than are currently reported. In addition, misdiagnosis of the disease is prevalent, increasing the impact of underreporting by an unknown factor. Almost 100,000 cases have been reported to the CDC from 1982-1996. During that time the reported incidence of the disease increased by at least 32 fold!

The disease has been reported in almost all states, although certain regions of the country are considered endemic, and some considered hyperendemic--there appear to be geographic clusters of higher infection. Infection varies from state to state, and even within states from county to county. While the surveillance of the disease leaves a great deal to be desired, two trends are clear--first, the incidence of the disease is increasing rapidly; and, second, there appears to be a spread of the organism to new areas.

LYMErix is a first generation, recombinant OSPa based vaccine. It has a unique mode of action for vaccines. It stimulates specific protective antibodies to be produced in the person vaccinated against OSPa, immunogenicity (the process of triggering protective antibodies). However, OSPa is not usually found in the human host, and it is thought that the bacteria changes its outer surface (triggered by heat) when moving from the tick vector to the human, this is called "upregulation" and "antigenic shifting."

So theoretically OSPa would not be a good candidate for a vaccine in humans, in whom OSPa is not generally found or detectable. It is thought that the mechanism of action is that when a tick bites an infected host and consumes a blood meal where the blood contains antibodies to OSPa that these antibodies kill the bacteria in the midgut of the tick where OSPa is not only detectable, but the dominant outer surface protein. Thus, the bacteria are killed before transmission occurs. Theoretically.

I. EFFICACY ISSUES:

A. EFFICACY UNIMPRESSIVE/OVERSTATED:

The commercials (that we have all seen) for LYMERix state that the vaccine, like all vaccines, may not be 100% effective. That much is true. How effective is the vaccine? The commercial, the literature and news reports have cited an 80% efficacy rate in preventing "definite" and "aymptomatic" lyme disease. However, if you include the category of "possible" lyme disease as well, the overall efficacy rate is 68%. If you include the category of "unconfirmed" lyme disease, in which the vaccine has negative efficacy, or some percentage of those cases, the efficacy rate is even lower, closer to 50%. The reported efficacy figures depend upon a semantic/definitional game--by creating different categories of vaccinees for the statistics Smith Kline has hidden the overall poor efficacy of this vaccine. The category of "unconfirmed" cases is the best example. The vaccine had negative efficacy in these categories, and by excluding this category they have effectively artificially inflated the efficacy numbers--a disturbing number of people got sick with something but due to

the absence of laboratory confirmation Smith Kline did not count any of these people as having gotten lyme, though they may have had every symptom. Does this make sense when lyme is a clinical diagnosis? And, perhaps this tells us something about people who don't develop a rash, or detectable antibodies. Perhaps the vaccine is altering the natural presentation of the disease. These unimpressive rates are only achieved after the third shot, now schedulled a year after the first shot is taken. Accelerated dosing schedules are in trials, but reliable data has not been reported. (See tables 1 and 2).

It is interesting to note that the Smith Kline study did prove that lyme disease is hyperendemic, and seriously underreported. Vaccine recipients live(d) in endemic areas. The group receiving vaccines and placebos showed per capita infection rates in the study exceeding 1000 per 100,000, both in year 1 and year 2, even following vaccination!

Another interesting tidbit arose from the study. While the data has not been published it has been presented showing that 35-40% of people in both the Smith Kline study and the Connaught study who developed lyme disease with confirmation by PCR testing and/or culture, were negative by conventional serologic antibody testing. In addition, there is obviously an additional percentage of people without any laboratory evidence of lyme disease, who contracted the disease--given the problems with all lyme testing, a known population of seronegative patients, and the fact that the diagnosis of lyme is ultimately a clinical one given the unreliability of the testing.

B. EFFICACY ONLY ACHIEVED AFTER 3 SHOTS:
After only 2 shots the efficacy rates are even less impressive, 57% in preventing "definite" and "asymptomatic" lyme disease. If you include the category of "possible" lyme disease the overall efficacy rate is only 46%. If you include the category of "unconfirmed" lyme disease, in which the vaccine has negative efficacy, or some percentage of those cases, the efficacy rates are even lower than that. So after shot 1 and shot 2 and before getting shot 3 the benefits of the vaccine are especially dubious, especially when weighed against safety concerns. Until the third shot then, currently schedulled a year later, efficacy is unimpressive, dubious at best. After the third shot efficacy rates improve, however, protective antibodies begin to diminish quickly and we know that a year after shot 3 (whenever given--even on accelerated schedule) they have fallen to close to where they are after 2 shots, to unimpresive levels of protection. (See table 2).

C. DURATION OF PROTECTION UNCERTAIN/LIMITED:
We know that the protection conferred after 3 shots does not last, and Smith Kline has reported that protective antibody levels drop to the level achieved after 2 shots in less than a year after the third shot. So whatever protection is conferred, diminishes quickly. So additonal booster shots will definitely be needed, but the safety, efficacy and timing of such shots has not been studied, tested or approved so safety and efficacy of additional shots, which is of concern (additonal shots may trigger problems, aggravate problems from previous shots). No FDA approval has been sought or obtained for additonal shots. Data is being reported anecdotally only by Smith Kline. They may not seek FDA approval for boosters, instead relying on doctor's right to use approved medications for "off label," (unapproved) uses.

D. BOOSTERS WILL BE NEEDED/SAFETY/EFFICACY/TIMING NOT STUDIED/NOT APPROVED BY FDA:
As stated above, additional booster shots will be necessary following the 3rd shot. We do not know the optimal timing, safety or efficacy of such shots, and such shots have not been approved by the FDA. Safety and efficacy issues remain, and are potentially more dramatic than the same issues after 3 shots only. Safety concerns include additional boosters causing, aggravating problems caused by the first 3 shots and perhaps remaining undetected. A third booster could overwhelm the immune system, or overcome tolerances to side effects that lasted through the first 3 shots. Nor do we know whether additional boosters will provide the same level of protection as the 3rd shot does. Why, knowing that boosters would be needed, did Smith Kline seek approval based on studies that did not address these issues? Perhaps they are aware of problems, frightened by what formal data may show? The unanswered questions abound.

E. VACCINE DOES NOT PROTECT AGAINST ALL STRAINS OF LYME:
Lyme disease, caused by the bacteria Borrelia Burdorferi, has exhibited genetic variations, known as strains. While there are three major strains of lyme disease found in the world, there are substrains by the hundreds. Limited research has been done but strain variation has been associated with variant symptomatic presentation. In the United States where one major strain is found, almost 300 variant substrains have been identified. Smith Kline states that the vaccine has not showed substantial variability in efficacy against strains tested. How many strains have they tested? What safety issues are involved with variant strains and the vaccine? Connaught, which is developing a recombinant OSPa vaccine, similar to that which Smith Kline is marketing, has recently entered into an agreement with MedImmune to co-develop a vaccine based upon DbPa, to address the problems of strain variation. The reports from MedImmune indicate that OSPa is not protective against many "wild" strains found in the field. It is thought that DbPa may protect against most/all strains. Whether this is true or not, it is clear that OSPa is problematic in this regard. Also, Smith Kline and Pasteur Meriux Connaugt (PMC) were in a race to market with their OSPa vaccines for several years. One has to wonder why Connaught has not sought final approval for their OSPa vaccine? The studies have been finished for a long time. No new data is being collected in trials. Has PMC abandoned their OSPa vaccine because of safety and efficacy issues, not to mention lawsuits arising out of the trials? Does the deal between PMC and MedImmune indicate that PMC recognizes that OSPa vaccine is a failure (although maybe a good first step in developing a vaccine that will be safe and effective in the future).

F. VACCINE NOT EFFECTIVE IN PEOPLE > 70 YEARS OF AGE:
Older people were included in the study but the vaccine proved to be less effective in them than 15-69 years old so the vaccine was not approved for use in this age group. It is uncertain why this variant result occurred.

G. EFFICACY RATES MAY HAVE BEEN INFLATED BY AWARENESS:
Those who participated in the trials were obviously aware of, and concerned about lyme disease (or they wouldn't have volunteered for the trials--they were not paid). Part of the study design called for vaccine recipient education--thus these people may have taken more precautions than the ordinary person, thus lowering infection rates overall. H. WERE ADVERSE EVENTS FAIRLY REPORTED/DOCUMENTED: Both the Smith Kline and Connaught trials resulted in the filing of a number of lawsuits. One of those suits alleged, among other things, that adverse events were not promptly and honestly reported to the FDA. Anecdotally, many participants who developed illness reported that the doctors involved in the studies were dismissive of their complaints, rather than thorough in evaluating the question of whether an event was related or unrelated to vaccination.

II. SAFETY ISSUES:

A. PEOPLE WITH A PRIOR HISTORY OF LYME MAY BE AT RISK:
This may be the most serious safety issue associated with the vaccine. The target group for this vaccine is people who live in endemic areas. We know that these areas feature high incidences of infection, including "asymptomatic" infection, undiagnosed infection, and misdiagnosed infection. It is impossible because of the unreliability of laboratory testing to screen candidates for the vaccine for lyme disease effectively. People with a recent history of lyme were excluded from the study. People with a more remote history of simple infection were included, but comprised only a small percentage of people in the study (total of 11% self-reported prior history--only 2% with serologic evidence for their prior lyme). These people were not studied as a seperate high risk group but the Smith Kline study does admit that people with a prior history of lyme did suffer from a higher incidence of adverse effects from the vaccine. These side effects were greater in number following the 2nd and then the 3rd shot. What the effect of additional booster shots will be is unknown. Analytically it is obvious that the study design, and data reported, deliberately glossed over this vital safety issue.

B. VACCINE NOT SAFE FOR CERTAIN TISSUE TYPES:
Two tissue types, HLA DR2 and HLA DR 4 have been specifically associated with a risk for chronic, destructive arthritic symptoms caused by lyme. These seem to be unresponsive to

"adequate" antibiotic treatment, even when initiated promptly. It is theorized that this is due to an autoiimune mechanism, triggered by the infection, and likely because of molecular mimicry--the bacteria shares certain genetic traits with our own tisse; antibodies formed to attack the bacteria, attack our tissue. Similar associations have been made with a smaller percentage of people with other tissue types as well. Recently a specific mechansim for this autommunity has been proposed, and documented, for people with the tissue type HLA DR4. There is a link between OSPa and this mechanism and it is feared that OSPa vaccination may trigger this process, even in the absence of bacterial infection. OSPa vaccination in animals has triggered severe destructive lyme arthritis. Studies of other tissue types relative to this concern have not been performed. Dr. Steere, principal investigator for the vaccine has expressed "concern" over this general issue. Additional booster shots may exacerbate the problem. The vaccine was approved without even a warning concerning this issue, and without instructions to screen candidates for tissue type and not vaccinate people with affected tissue types. Other tissue types may be at similar, or lesser risk--this is unknown. A number of lawsuits were filed against both Smith Kline and Connaught during the trials claiming adverse events. However, the study reports no such adverse events. What happened to those people? Anecdotal reports have been received about such events, and some are reported in the Lyme Alliance News Letter. Other information may be found on the Lymenet web site. If such incidents occur, report them there, and, even more important, make sure they are reported the the FDA Vaccine Adverse Events Reporting System (VAERS).

C. PEOPLE WITH OTHER HEALTH CONDITIONS MAY BE AT RISK:
People with health conditions including arthrtitic condtions, muscoloskeletal disorders, certain cardiac problems, neurologic problems, immunodeficiencies, a history of alcohol or drug abuse, and those receiving long term antibiotic treatment for any illness, along with those with hypersensitivity reactions to previous vaccinations were excluded from the study. So were those who had received treatment for lyme disease within three months of the study. Pregnant mothers were also excluded. Thus, the safety and efficacy of the vaccine in these groups has never been studied, and the vaccine cannot be said to be safe for them.

D. VACCINE NOT APPROVED FOR CHILDREN Children were not included in the original study and the vaccine has not been approved for use in children. Trials involving children are under way now. I would not permit a child of mine to participate.

E. VACCINE NOT EFFECTIVE IN PEOPLE > 70 YEARS OF AGE:
Older people were included in the study but the vaccine proved to be less effective in them than 15-69 years old so the vaccine was not approved for use in this age group. It is uncertain why this variant result occurred.

F. SHORT TERM FOLLOW UP/LIMITED STUDY:
Another serious issue involving study design is the short term follow-up of the study. Vaccinees were only followed during the study and then for less than a year afterwards. Thus, any mid to long term consequences of the vaccine, problems that might not be detected within the time frame of the study, could not have been recognized. Furthermore, while 11000 people were involved in the study, only half received the vaccine. It is very possible that adverse effects from the vaccine in the broader population might not have been detected. Remember that a serious adverse event that occurs to 1 in 1000 people sounds insignificant. However that figure translates into 1000 people in 1,000,000. Now it sounds more significant. One final note, the lack of long term follow up is of great concern in a disease that may become latent and then reemerge later as lyme is known to do.

III. OTHER ISSUES/CONCERNS:

A. VACCINE DOESN'T PROTECT AGAINST ALLCASES OR OTHER TICKBORNE DISORDERS/DON'T ABANDON OTHER PROTECTIVE MEASURES OR GET A FALSE SENSE OF SECURITY:
Since the vaccine does not protect against all strains of the vaccine, or 100% of recipients, and since protective levels are lower following shots 1 and 2, nor does the vaccine protect against Babesiosis, Ehrlichiosis, Rocky Mountain Spotted Fever, Tick Born Encephalitis, or a variety of other less

common tickborne diorders spread by the bite of the same tick, it is vital that people in endemic areas not lower their vigilance with regard to other protective measures (i.e., property management, proper attire, personal repellents, and tick checks). A false sense of security could lead to serious consequences. These facts should be carefully explained to all vaccine recipients.

B. VACCINATION WILL CONFUSE ALREADY PROBLEMATIC TESTS MAKING DIAGNOSIS EVEN MORE DIFFICULT:
Because the vaccine itself will cause certan antibodies to be produced, the appearance of these antibodies will confuse existing testing geared to those antibodies--the tests will not be able to distinguish between antibodies caused by vaccination as opposed to those caused by infection. Rather than question people tested for lyme as to whether they have recieved the vaccine, the tests have been reconfigured to discount certain significant and unique antibody responses. Thus, already reliable testing has been rendered even less reliable--and this affects both those who recieve the vaccine, and those who don't. Diagnosis will be even more difficult, and people put at greater risk for a delay that worsens the prognosis for treatment. Prompt diagnosis and early treatment for proper duration, at proper dosage is essential in preventing sequelae of the disease. This will be more difficult now than it has been. Further, as mentioned above, a large number of patients in the vaccine trials developed illnesses that could not be confirmed as lyme. Perhaps this means that the vaccine alters the natural presentation of the disease, and perhaps the natural course of infection. We simply do not know without extensive additional study.

C. FDA/MEDICAL COMMUNITY HAS RESERVATIONS ABOUT LYMERix:
The vaccine was approved with a record number of reservations by the FDA and the approval came in record time, and in an atmosphere of pressure on the FDA to generally speed up their drug approval process. This atmosphere has been created by disease advocacy groups exerting political pressure, in particular , HIV/AIDS patients. Unfortunately, while HIV/AIDS patients have received new drugs in record time, the pressure has been applied across the board and numerous drugs have been approved but then pulled from the market recently due to safety issues.

IV. THE BOTTOM LINE:
Given all of the above it is apparent that the approval of the vaccine was premature. Dr. Steere, principal investigator for the vaccine, has expressed concern over its long and short term safety. The FDA approval came with a unique number of reservations and concerns. Other researchers have simply stated that this vaccine is not safe for human beings. Vaccines given to dogs have turned out to have previously undetected long term consequences, and have never been particularly effective. Dr. Steere himself has declined to receive his own vaccine. The vaccine is expensive ($60-80 per dose, with three doses needed in the first year or earlier, and boosters needed but no one knows when, or how often).

Study design glossed over the biggest safety issues with the vaccine. Anecdotal reports of adverse events are flowing in, but have been denied by Smith Kline, as they were during the trials, despite the fact that several ended up in litigation. All of the above data is based upon Smith Kline's own studies--there may be reason to doubt the accuracy of this data, especially because the integrity of many of the researchers has been questioned, and their aptitude for diagnosing lyme disease is a matter of some debate amongst patients and clinicians. There are at least two schools of thought when it comes to lyme disease issues; it is fair to say that the researchers involved represented only one school of those schools.

If there were a safe and effective vaccine, lyme advocates would be wholeheartedly endorsing this as an additional tool to add to the arsenal of protective measures available to guard against, or minimize the risk of contracting a potentially devastating illness. These advocates have no vested interest in preventing a good vaccine from getting to market. Smith Kline does have a vested interest in marketing the vaccine in which they have invested millions of dollars.

The dubious benefits conferred by vaccination with LYMErix are far outweighed by the known safety issues, and the many unanswered questions.

Table 1.
Case Definitions for Lyme Disease

Definite Lyme Disease
Any of the following clinical manifestations observed by the investigator and at least one confirmatory laboratory test. In subjects with erythema migrans, a photograph of the lesion was also required.

Clinical manifestations
Erythema migrans (an expanding red skin lesion, often with partial central clearing)
Neurologic manifestations (meningitis, cranial neuritis)
Musculoskeletal manifestations (with objective evidence of joint swelling in one or a few joints)
Cardiovascular manifestations (atrioventricular block)

Laboratory confirmation
Positive culture for *B.burgdorferi* from skin-biopsy sample
Positive PCR result for *B.burgdorferi* DNA from skin-biopsy sample, cerobrospinal fluid or joint fluid.
Seroconversion on Western blotting (defined as a negative result followed by a positive result)
Positive IgM blot--at least 2 of the following 3 IgM bands: 23kd (outer-surface protein C), 39kd, and 41 kd.
Positive IgG blot--at least 5 of the following 10 IgG bands: 18, 23, 28, 30, 39, 41, 45, 58, 66, and 93 kd

Laboratory-confirmed asymptomatic B. burgdorferi infection

No symptoms
IgG seroconversion on Western blotting between month 2 and month 12 in the first year or between month 12 and month 20 in the second year

Possible Lyme disease

Influenza-like illness--fever, fatigue, headache, chills, muscle aches, mild stiff neck or backache without cough, coryza, diarrhea or vomiting-- with IgM or IgG seroconverion on Western blotting
Physician-diagnosed erythema migrans lesions >5cm without laboratory confirmation

Unconfirmed Lyme disease

All suspected cases that could not be confirmed

Table 2.
Attack Rates of Lyme Disease and Vaccine Efficacy in the Study Population*

Lyme Disease	Year 1						Year 2					
	VACCINE (N=5469)		PLACEBO (N=5467)		P VALUE	VACCINE EFFICACY (95%CI)	VACCINE (N=5469)		PLACEBO (N=5467)		P VALUE	VACCINE EFFICACY (95%CI)
	No. of cases	Attack Rate	No. of cases	Attack Rate			No. of Cases	Attack Rate	No. of Cases	Attack Rate		
		%		%		%		%		%		%
Definite												
Erythema Migrans	21	0.38	41	0.75	0.01	49 (14 to	15	0.27	65	1.19		77(60 to

Erythema migrans	21	0.36	41	0.73	0.01	70)	15	0.27	65	1.15		87)
Neurologic Involvement	0	0	1	0.02			0	0	1	0.02		
Arthritis	1	0.02	1	0.02			1	0.02	0	0		
Carditis	0	0	0	0			0	0	0	0		
TOTAL Definite Cases	22	0.40	43	0.79	0.009	49 (15 to 69)	16	0.29	66	1.21		68 (53 to 78)
Asymptomatic												
Asymptomatic Infection	2	0.04	13	0.24	0.004	85 (32 to 97)	0	0	15	0.27	0.001	100 (26 to 100)
TOTAL Definite and Asymptomatic cases	24	0.44	56	1.02		57 (31 to 73)	16	0.29	81	1.48		80 (66 to 88)
Possible												
Influenza-like illness with seroconversion	13	0.24	17	0.31	0.46	24 (-57 to 63)	12	0.22	21	0.88		43 (-16 to 72)
Physician-diagnosed erythema migrans	7	0.13	9	0.16	0.61	22 (-109 to 71)	7	0.13	6	0.11	0.78	-17 (-247 to 61)
TOTAL definite, asymptomatic, and possible cases	44	0.80	82	1.50	0.001	46 (23 to 63)	35	0.64	108	1.98		68 (53 to 78)
Unconfirmed	515	9.42	468	8.56	0.12		339	6.20	326	5.96	0.61	

*CI denotes 95% confidence interval

- Tables 1 & 2 are taken from Steere, et al. Vaccination against Lyme Disease with Recombinant *Borrelia burgdorferi* Outer-Surface Lipoprotein A with Adjuvant [Original Articles] N Engl J Med 1998 Jul 23;339(4):209-215
- These tables are modified for the web. Errors are my own.

PRESENTATION FRIDAY APRIL 9, 1999
12TH ANNUAL LYME DISEASE FOUNDATION SCIENTIFIC CONFERENCE

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OspA Induces Lyme Arthritis In Hamsters
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Recently we presented evidence that adverse effects, particularly severe destructive Lyme Arthritis (SLDA) can develop in vaccinated hamsters after challenge with Borellia Burgdorferi sensu lato isolates. Hamsters were vaccinatee with whole-cell preparations of inactivated B. Burgdorferi sensu stricto isolates in alum. SDLA was readily evoked in vaccinated hamsters after challenge with

homologens or other B. Burgdorferi isolates. Arthritis was evoked before high levels of protective borreliacidal antibody developed or after the levels of protective antibody declined. We now show that vaccination with recombinant OspA, the vaccine against Lyme disease, can also induce SDLA. Hamsters were vaccinated withe 30, 60, or 120 mg or recombinant Osp A or an Osp A vaccine for dogs. Eleven days after vaccination with the recombinant Osp A, vaccinated hamsters were challenged in the hind paws with 10 (to the 6th power) B. Burgdorferi isolates 297 or C-1-11. Swelling was detected 7 days after infection, peaked on day 11 and gradually decreased. In addition, histologic evidence or erosive and destructive arthritis was demonstrated in the hind paws of Osp A vaccinated hamsters challenged with B. Burgodrferi. These findings demonstrate that vaccination with Osp A can induce adverse effects. Vaccination of humans with OspA should not be reccomended until the vaccine has been shown to be incapable of inducing SDLA.

ADDITIONAL LINKS:
Lyme Disease Human Vaccine Information (This is the first place to look!!!)
Smith Kline Prescribing Info
All About Lyme Disease Prevention (Smith Kline Site)

Lyme Groups On the Vaccine:
LDRC Phyllis Mervine On Lymerix
LymeNet - LymeNet Vaccine Position Paper
LymeNet - LymeNet Vaccine Frequently Asked Questions
LDA NJ Vaccine Position Paper
LDF Hope or Hype?
Sheller Ludwig & Badey, P.C. (Lymerix Class Action law firm website)
Complaint (class action complaint)
Correspondence -- NEJM 1997; 337: 794-795

Personal Stories/Experiences with the Vaccine:
Spotlight on Lyme, brought to you by the Lyme Alliance
lyme disease medical pages
Lyme Vaccine Hope or Hype?
Prevention1
Personal Stories of Lyme disease
Dear Dr Malloy
Vaccine Volunteer
Participant Questions Vaccine Safety
Lyme Disease Vaccine
A BCNEWS.com : Lawsuit Over Lyme Disease Vaccine Risks
Emerging Drugs and Devices - LYMErix
Consent form for vaccine trials

Important Medical And Journal Articles:
SDLA in Hamsters IAI -- Abstracts: Croke et al. 68 (2): 658
The Lyme Disease Vaccine: Conception..., Annals 18 Apr 00
O spA Induces Lyme Arthritis in Hamsters
JAMA Letters Lyme Disease Vaccine
Limitations of the OspA Vaccine for Humans: A Review
Clinical Discussion - Limitations of OspA Vaccine for Humans< /A>
Medimmune/PMC DBPa Vaccine
FDA APPROVES FIRST LYME DISEASE VACCINE
Recommenda tions for the Use of Lyme Disease Vaccine by the Advisory Committee on Immunization Practices (ACIP)
Correspondence -- NEJM 1998; 339: 1637-1639
NEJM Original Vaccine Article Abstracts & Editorial NEJM 1998; 339: 4
Steere OSPA Vaccine SKB Original Articles -- NEJM 1998; 339: 209-215
Sigal OSPA Vaccine PMC Original Articles -- NEJM 1998; 339: 216-222
Editorials Lyme Vaccine A First Step -- NEJM 1998; 339: 263-264